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A simple method for studying lacZ fusions in lacZ+ Escherichia coli hosts

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▼Genetic constructs with *lacZ* as a reporter gene fused to the regulatory sequences of other genes/operons are frequently used for studying the regulation of gene expression in response to genetic and environmental changes (Ref. 1). This is because the activity of its gene product, β-galactosidase, can be easily detected/assayed by using various chromogenic substrates, such as 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal; Ref. 2, 3). Studies involving the use of such constructs are normally carried out in *lacZ*$^-$(mostly Δ*lac*) hosts in order to avoid interference from the host β-galactosidase. This often requires additional steps in strain construction which is time-consuming and, at times, undesirable. We report here a simple method based on the so-called 'glucose effect' or catabolite repression (Ref. 1), that permits the use of *lacZ*$^+$ *Escherichia coli* hosts for studying β-galactosidase production from *lacZ* fused to other promoters. We find that the addition of glucose (0.4%) to X-gal-containing LB (Luria-Bertani medium) plates (Ref. 2) considerably reduces β-galactosidase production from the host *lac* operon without affecting that from *lacZ* fused to a non-catabolite repressible promoter. Even cleaner results are obtained when M9 minimal medium plates (Ref. 2) (hereinafter referred to as GM9 plates) containing glucose (0.4%) as the sole carbon source and other necessary supplements (and X-gal) are used. On both these plates, *Lac*$^+$ hosts give white colonies while their derivatives containing the *lacZ* fusion construct give blue colonies>. For the purpose of our studies, we have made use of a translational fusion construct of *gicA*, a gene which conditionally regulates cell growth at low temperatures (see note below), with *lacZ*. The fusion construct was cloned in a low copy vector, pACYC184 (Ref. 4), encoding resistance to chloramphenicol (Cm). Our findings are incorporated into the following protocol.

Protocol

In order to compare the results obtained with *lacZ*$^+$ and *lacZ* hosts using our method, pACYC184 and a derivative of this plasmid harbouring the *gicA':'lacZ* fusion (called pVSWT184), were independently used to transform two (wild-type) *lacZ*$^+$ *E. coli* strains, W3110 (Ref. 5) and MD2223(HfrC) (Ref. 6), as well as a *Lac*$^-$strain, TB-1 (Ref. 7). Prior to plating out, the transformation mixtures were each incubated in LB broth containing 0.4% glucose for ~45 min at 37°C. Cm-resistant transformants were selected on LB plates containing 0.4% glucose, X-gal (40 μg/ml) and Cm (12.5 μg/ml). Following incubation at 37°C for ~16–18 h, the blue pVSWT184 transformants could be clearly distinguished in all cases from the white pACYC184 transformants. These results were confirmed by screening independent clones of each type using 'spot-tests' For this purpose, two independent pVSWT184 transformants and one pACYC184 transformant (of each culture), were inoculated in LB broth containing 0.4% glucose and Cm and grown at 37°C to a density of ~2×10^8 cells/ml. Subsequently, each was diluted 100-fold in saline and 10 μl (~2×10^4 cells) of this diluted culture 'spotted' on the following plates: (1) LBXC (LB plates containing X-gal and Cm), (2) LBXCG (LB plates containing 0.4% glucose, X-gal and Cm) and (3) GM9proXC [GM9 plates containing 40 μg/ml proline (required for TB-1), X-gal and Cm (Ref. 7)]. After the 'spots' had dried, the plates were incubated at 37°C for ~ 16–18 h.

The results of our spot-tests (Fig. 1a) show that on the LBXCG plates, the spots of the pACYC184 (control)

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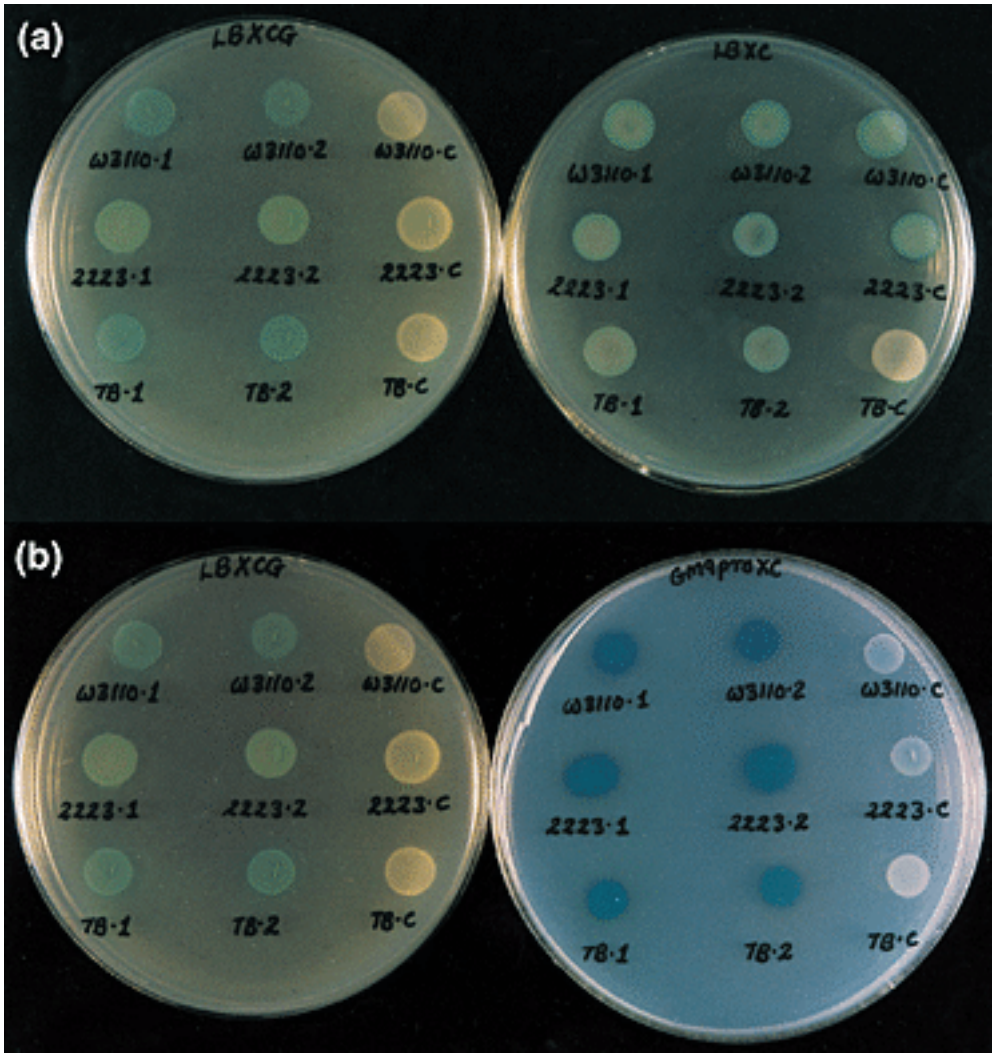

FIGURE 1. (a) Comparision of the results obtained using LBXCG (left) and LBXC (right) plates. (b) Comparision of the results obtained using LBXCG (left) and GM9proXC (right) plates (see text for details). In both the figures, the order of the spots from left to right and top to bottom, is as follows. First row: two clones of W3110/pVSWT184 and one clone of W3110/pACYC184; second row: two clones of MD2223/pVSWT184 and one clone of MD2223/pACYC184; third row: two clones of TB-1/pVSWT184 and one clone of TB-1/pACYC184.

transformants of the *lacZ*$^+$ strains (like that of the *Lac*$^-$ strain, TB-1) are white, whereas the spots of all the pVSWT184 (fusion) transformants (as expected) are blue. However, on the LB plates without glucose (LBXC), hardly any colour difference is seen between the fusion and control transformants, in the case of *lacZ*$^+$ strains. The results obtained with the LBXCG plates are also found to be quite comparable with those obtained with the GM9proXC plates (Fig. 1b). It must be pointed out, however, that for clean, unambiguous results, the time of incubation for either of these plates should not exceed 18 h (at 37°C).

Our results show that the use of LB plates containing glucose (and X-gal) permits the use of *lacZ*$^+$ *E. coli* hosts for studying *lacZ* fusions, with even cleaner results being obtained by using X-gal-containing GM9 plates. However, our method has three limitations: (1) it cannot be used for fusion constructs made using catabolite-repressible promoters, (2) it cannot be used in cases where the host *lac* promoter itself is not catabolite repressible [e.g. *lacUV5* promoter (Ref. 1, 2)] and (3) although this procedure works well for fusion constructs cloned on multicopy plasmids (including 'low-copy'-number plasmids), there is still sufficient basal expression of *lacZ* such that it may not work equally well for some single-copy chromosomal fusions.

Note

A new gene, *gicA*, whose product conditionally regulates the growth as well as streptomycin resistance at low

temperatures, of *E. coli* K-12 strains, has been identified, mapped, cloned and sequenced in our laboratory [S.H. Mangoli *et al*. (1993) XVIIth Intl Congress of Genetics and Y. Ramanathan (1995) Molecular genetics of growth of *E. coli* in cold. (PhD Thesis, University of Bombay)]. The sequence of this gene has also been submitted to GenBank (Acc. No. L29054). Our unpublished results further show that *gicA* is expressed well both at 37°C and at lower temperatures.

Materials

The suppliers of chemicals were as follows Glucose (E. Merck), X-gal (Boehringer Mannheim), chloramphenicol (Sigma), proline (Sigma), LB medium components [Bacto-yeast extract (Difco Laboratories), Bacto-tryptone (Difco Laboratories), agar (Difco Laboratories)], M9 medium components (Sigma). For details of LB and M9 minimal medium preparation, see Miller (Ref. 2).

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Products Used

glucose: glucose from Merck
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glucose: glucose from Merck
X-gal: X-gal from Boehringer Mannheim
X-gal: X-gal from Life Technologies (Gibco BRL)
Chloramphenicol: Chloramphenicol from Sigma
proline: proline from Sigma
Yeast extract: Yeast extract from Difco
Yeast extract: Yeast extract from Difco
Bacto-tryptone: Bacto-tryptone from Difco
agar: agar from Difco